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Effect of probiotics (Binifit™) on survival, growth, biochemical constituents and energy budget of the freshwater prawn *Macrobrachium rosenbergii* post larvae

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ABSTRACT

The present study was conducted to investigate the effect of probiotics, Binifit™ on survival, growth, biochemical constituents and energy budget of the freshwater prawn *Macrobrachium rosenbergii* post larvae (PL). The basal diet was prepared by the supplementation of probiotics Binifit™ at four different concentrations (0.5%, 1%, 1.5% and 2%) was incorporated. Feed without Binifit™ served as control. These feeds were fed to *M. rosenbergii* PL for a period of 60 days. Leaching of these diets varied between 13.7%-15.0% at time duration of 8 hrs. The growth parameters, such as survival, weight gain, specific growth rate, feed conversion efficiency and protein efficiency rate were significantly ($P<0.05$) higher in 2% Binifit™ incorporated diet fed PL followed by other experimental groups when compared with control. On the other hand, the food conversion ratio was significantly ($P<0.05$) lower in 2% Binifit™ supplementation diet fed PL. This indicates the fact that this diet had resulted in higher growth rate than that of other experimental diets. Similarly the proximate composition of the total protein, amino acid, carbohydrate, and lipid content were significantly ($P<0.05$) higher in 2% Binifit™ incorporated diet fed PL. However, insignificant differences were recorded in moisture content between control and experimental groups. The energy utilization parameters, such as feeding rate, absorption rate, conversion rate, excretory rate and metabolic rate were significantly ($P<0.05$) higher in 2% Binifit™ supplementation diet fed PL. However, Binifit™ supplementation diet fed PL resulted in better growth performance. This is only because of presence of Binifit™ in the feed. Therefore, incorporation of this probiotics in aqua feed is stressed for promoting sustainable culture of *Macrobrachium*.

Introduction

Giant freshwater prawn, *M. rosenbergii* (de Man) has been regarded as prime aquaculture prospect in many countries. It is commonly called 'Scampi', has been found to be more suitable for freshwater aquaculture. This species of prawn have remarkable advantage, because of its omnivorous feeding habit, fairly high growth rate, attractive size, better meat quality with good amount of protein, tolerance of wide range of temperature (15-35ºC) and resistance to diseases (Ling, 1969). The use of probiotics in the aquatic organisms is increasing along with the demand for more environment-friendly aquaculture practices (Gatesoupe, 1999). Several mechanisms have been suggested as modes of action for probiotic bacteria. The competitive exclusion, based on the removal of the pathogen by the beneficial population, has been regarded as important by many authors (Gatesoupe, 1999). Some studies have attributed the enhancement of animal growth to the nutritional benefits of probiotic bacteria, such as vitamin production, a viability of minerals and trace elements and production of important digestive enzymes (Holzapfel *et al.*, 1998). These essential growth nutrients are beneficial for enhancing the optimum growth, also they could benefit to their invertebrate host by competitive exclusion against pathogens (Gomez-Gil *et al.*, 2000) or by increasing the host resistance and immunity (Uma *et al.*, 1999), which are beneficial to achieve higher survival rate and healthier animals. However, all of these proposed modes of action require that the potential probiotic is able to reach the location where the probiotic effect is required and is able to successfully colonize this region (Verschuere *et al.*, 2000). Probiotics are recently incorporated into feed basically to enhance immunity status and promote growth rate (Havenaar and Huis in'tveld, 1992). Venkat *et al.* (2004) has worked out different modes of administration of probiotics to *M.roesenbrgii* PL either through feed or as bioencapsulated in *Artemia*. Shinde *et al.* (2008) reported use of different commercial probiotics by administering different probiotic supplements through feed to PL of *M. rosenbergii*. Saad *et al.* (2009) have reported use of Biogen® as probiotics for *M. rosenbergii* PL. Therefore, this study attempted to investigate the effect of probiotics (Binifit™) on the survival, growth, biochemical constituents and energy budget performance of the PL of the freshwater prawn *M. rosenbergii*.

Materials and methods

The post larvae of freshwater prawn, *M. rosenbergii* (PL 15) were purchased from a Happy Bay Annexe, Kanchipuram, Tamilnadu, India and were stocked in a cement tank (1000 L) filled with freshwater. The PL were acclimatized at ambient laboratory conditions for 15 days (up to PL 30) and starved for 24 h before the commencement of the feeding experiment. The

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experimental water had these physicochemical parameters: pH 7; total dissolved solids 0.90 g/L^{-1}; dissolved oxygen 7.20 mg/L^{-1}; BOD 30.00 mg/L^{-1}; COD 125.00 mg/L^{-1}; ammonia 0.028 mg/L^{-1}.

Diet preparation

The composition of the experimental diets is given in Table -1. The probiotics Binifit^{TM} (Tablets, India Ltd) was incorporated in to the test diets at five different concentrations individually 0% (control), 0.5%, 1%, 1.5% and 2% respectively. Feed formulation was done basically by ''Pearson's square-method'' using determined values of 45% protein content (Table-1). The proportion of each ingredient required was calculated precisely providing allowance for the premix. The dough was steam cooked and cooled to room temperature. After that different the concentration of Binifit^{TM} was mixed with the dough and the feeds were pelletized separately with a locally made (Kolkata, India) hand pelletizer. The pellets were dried in a thermostatic oven (M/s Modern Industrial, Mumbai, India) at 40^0 C until it reached constant weight and stored in airtight jars at room temperature. The biochemical constituents of the experimental diets were determined, total protein (Lowry *et al*., 1951), amino acid (Moore and Stein, 1948), lipid (Folch *et al*., 1957), carbohydrate (Roe, 1955), ash and moisture contents (APHA, 2005). These diets were freshly produced after 30 days to ensure high probiotic viability throughout the duration of feeding trail. In the control diet, no Binifit^{TM} was found throughout the duration of feeding trail.

Water stability of experimental feed

The water stability of the prepared diets was tested over a period of 8 hrs by following standard method (Immanuel *et al*., 1997). 1g of diet was soaked in glass bowls (capacity-100cc) with three replicates. They were then kept immersed separately in plastic troughs containing 10 liters of water for a period of 4, 6 and 8hrs separately. After stipulated duration, water from each bowl was drained carefully using No. 30 blotting silk cloth and the residue was dried in a hot air oven at 105^0 C for 30 min., followed by further drying at 65^0 C until to reach constant weight. The mean weight before immersion and after drying was used to calculate the percentage dry matter loss, which is the measure of the water stability of pellets for the corresponding time intervals. Finally mean percentage of leaching of dry matter was estimated.

Feeding experiment

M. rosenbergii (PL-30) with the length and weight range of 1.34±0.20 cm and 0.18±0.04g respectively were used for feeding experiment. 40 PL for each feed in triplicate were maintained in plastic tanks with 20 L water. The PL was maintained at the stocking density of 2/l. One group served as control, which devoid of probiotics (0%). The experimental groups were fed with the respective concentration of Binifit^{TM} incorporated diets. The feeding was adjusted to two times a day (6:00 am and 6:00 pm). The daily ration was given at the rate of 10% of the body weight of PL with two equal half throughout the experimental period. The unfed feed, faeces and moult if any, were collected after the respective hours of feeding. The feeding experiment was prolonged for 60 days; mild aeration was given continuously in order to maintain the optimal oxygen level.

Determination of food indices

After the feeding trial, the food indices parameters such as survival (S), weight gain (WG), specific growth rate (SGR), feed conversion rate (FCR), feed conversion efficiency (FCE) and protein efficiency rate (PER) were individually determined by following equations.

Survival (%) = Total No. of live animals/Total No. of initial animals x 100

Weight gain (g) = Final weight (g) – Initial weight (g)

Specific growth rate (%) = log w_2 – log w_1/ t x100 (where, w_1 & w_2 = Initial and Final weight respectively (g), and t = Total number of experimental days)

Feed conversion rate (g) = Total Feed intake (g)/ Total weight gain of the prawn (g)

Feed conversion efficiency (%) = Biomass (g)/ Total Feed intake (g) x 100

Protein efficiency rate (g) = Total Weight gain of PL (g)/ Total Protein consumed (g)

Energy budget

The energy content of whole prawns, feeds, moult and faeces was measured using Parr 1281 Oxygen Bomb Calorimeter. The energy budget was calculated using the equation (C = (P+E) + R + F +U) derived by Petrusewicz and Macfadyen (1970); where, C is the energy consumed in food; P is the growth; R is the material lost as heat due to metabolism; F is the energy lost in faeces; U is the energy lost in excretion and; E is the energy lost in exuvia.

$$\text{Feeding Rate (FR)} = \frac{\text{Mean Food Consumption (k.cal/day)}}{\text{Initial live weight of the prawn (g)}}$$

Mean Absorption = Mean Food Consumption (k.cal/day) – Mean Food Excreted as Faeces (k.cal/day)

$$\text{Absorption Rate (AR)} = \frac{\text{Mean Absorption (k.cal/day)}}{\text{Initial live weight of the prawn (g)}}$$

Mean Conversion = Mean weight gain (k.cal/day) + Mean exuvial weight (k.cal/day)

$$\text{Conversion rate (CR)} = \frac{\text{Mean Conversion (k.cal/day)}}{\text{Initial live weight of the prawn (g)}}$$

$$\text{Mean } NH_3 \text{ Excretion (k.cal/day) } NH_3 \text{ Excretion Rate (ER)} = \frac{}{\text{Initial live weight of the prawn (g)}}$$

Metabolic Rate (MR) = Absorption Rate (k.cal/g/day) – Conversion Rate (k.cal/g/day) + NH_3 excretion Rate (k.cal/g/day)

Biochemical constituents of the experimental animals

The initial and final day of the experiment, the biochemical constituents of the experimental animals were determined. The biochemical constituents, such as total protein (Lowry *et al*., 1951), amino acid (Moore and Stein, 1948), lipid (Folch *et al*., 1957), carbohydrate (Roe, 1955), ash and moisture contents (APHA, 2005) of individual diet fed prawns were measured.

Statistical analyses

One way analysis of variance (ANOVA; SPSS, 13.0) was used to determine whether significant variation between the treatments existed. Difference between means were determined and compared by DMRT test. All the tests used a significance level of $P<0.05$. Data are reported as means ± standard deviations.

Results and discussion

Probiotics are defined as live microbial feed supplements that beneficially affect the host by the production of inhibitory

compounds, competition for chemicals and adhesion sites, immune modulation and stimulation, improving the microbial balance and thereby increase the growth of candidate species progressively (Fuller, 1989; Verschuere *et al.*, 2000). In the present study probiotics Binifit™ was evaluated in terms of their impact on the survival, growth, nutritional indices, biochemical constituents and energy utilization in the freshwater prawn *M. rosenbergii* PL.

Biochemical constituents of experimental diet

The biochemical constituents of Binifit™ incorporated diets are presented in Table-1. The level of total protein and lipid were found to be between 39.40-40.10% and 8.90-9.28% respectively. The level of total carbohydrate was found to be between 19.50-21.76% respectively. The levels of ash and moisture contents were between 12.00-14.00% and 9.10-9.90% respectively. The digestible energy of these diets was ranged from 3228.17-3296.86 k.cal/kg^{-1} (Table-1). Swamy (1995) indicated that the *M. rosenbergii* required 35-40% protein and 7-8% of lipid as optimum in the diet. Mitra *et al.* (2005) reported that the *M. rosenbergii* required 30-40% protein and 3-7% lipid as optimum in the diet. Raj (1993) pointout that the *M. rosenbergii* required 25-40% carbohydrate as optimum in the diet. Mitra *et al.* (2005) reported that the *M. rosenbergii* required 25-35% carbohydrate as optimum in the diet. In accordance with the above reports of several authors, in the present study, the protein supplemented diets as well as control diets were prepared with the optimum level of 40% protein, 20% carbohydrate and 9% of lipid for *M. rosenbergii* PL.

Water stability of experimental diet

In the present study, the leaching percentage of the diets prepared is provided in the Table-2. During 8 hours period of the test the leaching percentage of 15% was found to be the maximum in 2% Binifit™ incorporated diet. In other diets, the percentage of leaching was varied between 13.70 and 14.50%. From the Table -2 it was understood that the leaching of the diet was faster till 4 hours and further exposure didn't appreciably increase the leaching. Similarly, Immanuel *et al.* (1997) reported that the inclusion of fishmeal in formulated diets and the increase of fishmeal proportion have decreased the water stability. It has been reported that the stability of pelletized feed is influenced by different factors such as composition of the feed, nature of ingredients, types of processing and moisture content (Hastings, 1976; Kainz, 1977). The compounded pelletized feeds are high-energy nutritive packages; they should be stable in water with a low rate of disintegration such that they will remain available to fish/shrimp for long periods, without the loss of nutrients through leaching (Pereira, 1991). The high stability in pelletized feeds is considered as one of the major advantages of artificial feed over the natural feed. Feeds must be easy to assimilate, posses high nutrient value and high stability in water (Lobaeva 1959).

Growth performance

In the present study probiotics provided beneficial effects on growth of *M. rosenbergii* PL. The present results indicated that the prawns offered probiotics diets exhibited greater growth than the control diet. There were significant differences in growth ($P<0.05$) on variations between experimental and control diets fed groups.

Survival

The minimum survival of 75.00±2.550% was recorded in *M. rosenbergii* post larvae (PL120) fed with control 1 diet. *M. rosenbergii* post larvae fed on 2% signify supplemented diet showed the maximum survival of 90.00±2.50% postlarve fed on diets 0.5%, 1% and 1.5% Binifit™ had survival percentage between 80.00±2.50% and 85.00±2.50% (Table-3). One-way analysis of variance revealed that variations in the survival of *M. rosenbergii* post larvae fed with control and experimental diets was statistically significant ($F=15.60$, $P<0.05$). During the feeding trail, experiment the prawns were behaved normally without cannibalism. Therefore, the probiotic diets fed prawns were resulted in significantly higher survival rate when compared with control group. In accordance with the present findings, Fernandez *et al.* (2011) reported the enhanced survival rate (92 to 98%) by the probiotics (Lactic acid bacteria) diets fed juveniles of *P. indicus.* Boonthai *et al.* (2011) stated that the black tiger shrimp, *P. monodon* fed with probiotic (*Bacillus* sp) supplemented diets was found to have maximum the survival rate up to 91.68%. Supportively, Saad *et al.* (2009) reported that different concentration of Biogen® (1%, 2%, 3% and 4%) supplemented diets had the better survival performance in *M. rosenbergii* PL, when compared to the control. Also, Venkat *et al.* (2004) pointed out that the survival performance of *M. rosenbergii* PL supplemented with *L. acidophillus* and *L. sporogenes* diets had 100% survival. Furthermore, Lara-Flores *et al.* (2003) reported that probioitics *S. faecium* and *L. acidophilus* (0.1%), and the yeast *S. cerevisiae* (0.1%) incorporated diets had significantly improved the survival (85.18 to 96.29 %) of Nile tilapia, *Oreochromis niloticus*.

Weight gain (WG)

The weight gain (WG) in the overall experimental period shows that 2% Binifit™ supplemented diet fed group has highest value of 1.04±0.10 and lowest value of 0.600±0.06 g in control diet fed group. Whereas the other diet fed prawns (0.5%, 1% and 1.5% Binifit™ supplemented diets) have more value of (0.70±0.05, 0.80±0.10 and 0.88±0.07g) weight gain. One way analysis of variance revealed that the variation in weight gain of *M. rosenbergii* between control and experimental diets were statistically significant ($F=13.78$, $P<0.05$). Similar results on significant improvement in weight gain (ranged from 99.48 to132.52%) was recorded by bioencapsulated of *L. acidophillus* (70 x10^7 cfu cells) and *L. sporogenes* (6x 10^7 cfu cells) supplemented diets fed *M. rosenbergii* PL (Venkat *et al.*, 2004). Likewise, Hisano *et al.* (2008) reported that probioitics *Saccharomyces cerevisiae* (2.0%) and yeast derivatives (2.0%) supplemented diets had increased the weight gain (ranged between 0.93 to 1.37g) of juvenile, *M. amazonicum*. It has been reported that the increase in weight gain was achieved by *M. rosenbergii* fed with bio-encapsulated diet containing *L. ceremoris* (Suralikar and Sahu, 2001). Also, Hidalgo *et al.* (2006) noted that probioitics *Bacillus toyoi* (0.5, 1 and 2 g kg^{-1}) and *B. cereus* (0.5, 1 and 2 g kg^{-1}) incorporated diets had improved the weight gain of juvenile dentex, *Dentex dentex*. Lara-Flores *et al.* (2003) reported that probioitics *S. faecium* and *L. acidophilus* (0.1%), and the yeast *S. cerevisiae* (0.1%) incorporated diets had improved the weight gain of Nile tilapia, *Oreochromis niloticus*

Specific growth rate (SGR)

The specific growth rate of the experimental diets fed prawns ranged from 0.669±0.034 to 0.880±0.026%. One way analysis of variance revealed that influence of Binifit™ supplementation on SGR of *M. rosenbergii* PL fed with control and experimental diets was statistically significant ($F=21.35$, $P<0.05$). A similar result on significant improvement in specific growth rate (1.14 to1.41%) was recorded by *L. acidophillus* and

L. sporogenes supplemented diets fed *M. rosenbergii* PL (Venkat *et al*., 2004). Likewise, Hernandez *et al*. (2009) reported that *Poecilopsis gracilis* fed with bio-encapsulated *L. casei* (0.7x 10^8 cfu ml^{-1}) has significantly increased specific growth rate (2.57 and 2.64%). It has also been reported that the significantly improved the specific growth rate (0.508 to 0.900%) was recorded by ornamental fishes (*Poecilia reticulate, Poecilia sphenops, Xiphophorus helleri,* and *Xiphophorus maculatus*) fed with *B. subtilis* (5 x10^8, 5x10^7, 5x10^6 and 5x10^5 cells g^{-1}) supplemented diets (Ghosh *et al*., 2008). Hidalgo *et al*. (2006) noted that probioitics *Bacillus toyoi* (0.5, 1 and 2 g kg^{-1}) and *B. cereus* (0.5, 1 and 2 g kg^{-1}) incorporated diets had improved the specific growth rate of juvenile, *Dentex dentex*. Also, Lara-Flores *et al*. (2003) reported that probioitics *S. faecium* and *L. acidophilus* (0.1%), and the yeast *S. cerevisiae* (0.1%) incorporated diets had improved the specific growth rate of Nile tilapia, *Oreochromis niloticus*.

Feed conversion ratio (FCR)

The post larvae fed with control diet had maximum (3.18±0.50g) of FCR. The prawns fed with 2% Binifit™ diet had the minimum 1.57±0.80g of FCR, followed by the individuals fed with 0.5% Binifit™ (2.75±0.27g), 1% Binifit™ (1.88±0.24g) and 1.5% Binifit™ (1.77±0.20g) (Table-3). One way analysis of variance revealed that the variation in FCR of *M. rosenbergii* between control and experimental diets were statistically significant (F=19.93, P<0.05). Similar results on food conversion ratio (2.21 to 2.75g) was noticed in bioencapsulated of *L. acidophillus* (70 x10^7 cfu cells) and *L. sporogenes* (6x 10^7 cfu cells) supplemented diets fed *M. rosenbergii* PL (Venkat *et al*., 2004). It has also been showed that 1%, 2%, 3% and 4% of Biogen® supplemented diets had significantly lowered the food conversion ratios of *M. rosenbergii* PL when compared to the control prawn (Saad *et al*., 2009). Also, Hisano *et al*. (2008) reported that probioitics *S. cerevisiae* (2.0%) and yeast derivatives (2.0%) supplemented diets had lower the food conversion ratio (2.93 to 3.13g) of juvenile, *M. amazonicum*. Merrifield *et al*. (2009) pointed out that probioitics *Bacillus subtilis* (7.79 log cfu g^{-1}), *Bacillus licheniformis* and *Enterococcus faecium* (8.05+8.23 log cfu g^{-1}) incorporated diets had significantly lower the food conversion ratio (0.85 to 0.90g) of rainbow trout, *Oncorhynchus mykiss*.

Feed conversion efficiency (FCE)

The control diet has lowest FCE of 0.94±0.02% and those fed with 2% Binifit™ supplemented diet has highest FCE of 1.44±0.10%. Whereas it was 1.26±0.03, 1.17±0.04 and 1.06±0.05% in 1.5, 1% and 0.5% diets respectively (Table-3). One way analysis of variance revealed that the variation in FCE of *M. rosenbergii* between control and experimental diets were statistically significant (F=88.25, P<0.05). Similarly Boonthai *et al*. (2011) showed that *P. monodon* fed with probiotic (*Bacillus* sp) supplemented diets had enhanced the feed conversion efficiency (1.78 to 1.86g) of PL of shrimp. Li *et al*. (2005) reported that brewers yeast supplemented diets had improved the FER of Juvenile hybrid striped bass, *Morone chrysops- x- M. saxatilis*.

Protein efficiency rate (PER)

After the feeding trail experiment of 90 days, the PER of post larvae *M. rosenbergii* was high (1.28±0.14g) in 2% Binifit™ incorporated diet. The *M. rosenbergii* fed with control diet had the minimum (0.826±0.042g) of PER. Where as it was 0.941±0.032, 1.04±0.18 and 1.12±0.19g in 0.5% 1% and 1.5% Binifit™ supplemented diets respectively (Table-3). One-way analysis of variance revealed that the variation in PER of *M. rosenbergii* between control and experimental diets were statistically significant (F=493, P<0.05). Similarly, Saad *et al*. (2009) reported that different concentration of Biogen® (1%, 2%, 3% and 4%) supplemented diets had significantly increased the protein efficiency rate (0.47 to 1.67g) of *M. rosenbergii* PL. Al-Dohail *et al*. (2009) suggested that 3.01x10^7 colonies/g of *L. acidophilus* incorporated diets had improved the protein efficiency rate (2.57g) of African Catfish, *Clarias gariepinus* fingerling. Supportively, Merrifield *et al*. (2009) reported that probioitics *B. subtilis* (7.79 log cfu g^{-1}), *B. licheniformis* and *Enterococcus faecium* (8.05+8.23 log cfu g^{-1}) incorporated diets had significantly improved the protein efficiency rate of rainbow trout, *Oncorhynchus mykiss*. Also, Hidalgo *et al*. (2006) reported that probioitics *B. toyoi* (0.5, 1 and 2 g kg^{-1}) and *B. cereus* (0.5, 1 and 2 g kg^{-1}) incorporated diets had improved the protein efficiency rate (2.31 to 2.71g) of juvenile, *Dentex dentex*. Lara-Flores *et al*. (2003) stated that probioitics *S. faecium* and *L. acidophilus* (0.1%), and the yeast *S. cerevisiae* (0.1%) incorporated diets had remarkably enhanced the protein efficiency rate (1.140 to 3.380 g) of Nile tilapia, *Oreochromis niloticus*.

Biochemical constituents of experimental animals

The biochemical compositions of edible organism are important in nutritional point of view. According to Vijayavel and Balasubramaniam (2006), the nutritive values of crustaceans depend upon their biochemical constituents. Body composition is a good indicator of the physiological condition of an aquaculture organisms and easy to assess. The initial day of the prawn body concentration of the biochemical constituents, such as total protein, amino acid, carbohydrate, lipid, ash and moisture of post larvae was recorded as 27.40%, 14.30%, 7.22%, 3.46%, 8.00% and 83.10% respectively.

Protein and Amino acid

The protein and amino acid of the control diet fed *M. rosenbergii* were 58.001±2.40 and 26.80±1.64% this level was increased 64.12±2.60% & 38.10±2.14% in 2% Binifit™ supplemented diet fed prawns and in the remaining groups namely 0.5%, 1% and 1.5%, it varied from 60.10±2.80% & 33.42±1.48% to 62.38±2.14% & 37.26±1.98% (Table-3). One way analysis of variance revealed that variations in the protein and amino acid content as *M. rosenbergii* post larvae fed with control and experimental diets was statistically significant (F=2.68 & 23.37, P<0.05). In consonance, Fernandez *et al*. (2011) reported that Lactic acid bacteria enhanced the crude protein content in juveniles of *P. indicus*. Supportively, Saad *et al*. (2009) reported that 1-4% Biogen® supplemented diets had significantly increased the carcasses protein content (61.6 to 67.1%) of *M. rosenbergii* PL. Likewise, Venkat *et al*. (2004) stated the significant improvement in tissue total protein content (65.14 to 70.04%) in bioencapsulated *L. acidophillus* and *L. sporogenes* fed *M. rosenbergii* PL. Also, Yu *et al*. (2009) reported that *Bacillus* spp (0.15% and 0.30%) incorporated diets had not significantly increased the body composition of total protein content (72.55 to 73.84 %) in white shrimp, *L. Vannamei*.

Carbohydrate

The carbohydrate content averaged 17.88% and it ranged from 15.08±1.02 to 20.42±1.60% among the tested groups. One way analysis of variance revealed that variations in carbohydrate content of *M. rosenergii* post larvae fed with control and experimental diets was statistically significant (F=6.02, P<0.05).

A similar result on improvement in tissues total carbohydrate content (10.16 to 13.34%) in the tissues was higher in *L. acidophillus* and *L. sporogenes* supplemented diets fed *M. rosenbergii* PL (Venkat *et al*., 2004).

Lipid

The lipid level in the control diet fed group was 7.82±1.74%, whereas it was 9.60±1.28, 10.63±1.32, 12.10±1.18 and 13.02±1.64% in 0.5%, 1%, 1.5% and 2% Binifit™ supplemented diets fed prawns respectively. One-way analysis of variance revealed that variation in lipid content of *M. rosenbergii* post larvae fed with control and experimental diets was statistically significant (F=8.01, P<0.05). Similarly Saad *et al*. (2009) reported that 1-4% Biogen® supplemented diets had significantly increased the carcasses lipid content (7.35 to 9.85 %) of *M. rosenbergii* PL. Accordingly, Lacticacid bacteria supplemented diets fed *P. indicus* juveniles showed the like in tissues lipid accumulation. Yu *et al*. (2009) pointed out that *Bacillus* spp (0.15% and 0.30%) incorporated diets had significantly increased the body composition of total lipid content (6.04 to 7.09 %) in white shrimp, *L. Vannamei*. Accordingly, Merrifield *et al*. (2009) reported that probiotics *B. subtilis* (7.79 log cfu g^{-1}), *B. licheniformis* and *Enterococcus faecium* incorporated diets had improved the body lipid content (95.00 to 108.00 %) of rainbow trout, *Oncorhynchus mykiss*. Also, Hidalgo *et al*. (2006) reported that probioitics *B. toyoi* (0.5, 1 and 2 g kg^{-1}) and *B. cereus* (0.5, 1 and 2 g kg^{-1}) incorporated diets had significantly improved the body lipid content (ranged between 114.5 to 150.0 %) of juvenile, *Dentex dentex*.

Ash

The ash content of *M. rosenbergii* fed with control diet was minimum (12.20±1.20%) followed by 0.5% (14.60±1.40%), 1% (16.40±1.60%), 1.5% (17.90±1.80%) and 2% (19.80±2.00%) diets fed groups (Table 3). One way ANOVA showed that, the variance in ash content among the diets was statistically significant (F=7.20, P<0.05). Similarly, lactic acid bacteria fed juvenile of *P. indicus* showed increased carcasses ash content (Fernandez *et al*., 2011). Similarly, El-Haroun *et al*. (2006) reported that different concentration of probioitics Biogens® (0.5, 1, 1.5 to 2.0%) supplemented diets had improved the whole carcass ash content (5.10 to 6.73 %) of Nile tilapia, *Oreochromis niloticus*. In contradictory to the above, Venkat *et al*. (2004) reported that the carcasses ash content was not increased with probiotic supplementation. Also, Hernandez *et al*. (2009) reported that in *Poecilopsis gracilis* fed with bio-encapsulated *L. casei* ($0.7x\ 10^8$ cfu ml^{-1}) has not significantly increased ash content.

Moisture

The postlarvae fed with control diet had maximum moisture content of 77.40±3.00% and displayed the minimum of 75.00±2.60% in 2% Binifit™ supplemented diet fed group. In remaining diets fed groups, it was 77.10±2.80 (0.5%), 77.00±2.90% (1%) and 76.50±3.20% (1.5%) respectively (Table-3). One way ANOVA showed that, the variance in moisture content among the diets was statistically non significant (F=<1, P>0.05). Supportively, Fernandez *et al*. (2011) assessed the moisture content of lacticacid bacteria supplemented diet fed PL of *P. indicus* was not exhibited significant variation when compared to control. Also, Venkat *et al*. (2004) reported that the tissues moisture content (75.35 to 75.75 %) of *L. acidophillus* and *L. sporogenes* supplemented diets as well as control (75.66 %) diet fed prawns were more or less same. Yu *et al*. (2009) reported that *Bacillus* spp (0.15% and 0.30%) incorporated diets fed in white shrimp, *L. Vannamei* had the moisture content of 76.25 to 76.95%, the same level of moisture content (76.86%) was recorded in control shrimps too.

Energy utilization performance

In living tissues, the bulk of energy is derived from the oxidation of the three main classes of foodstuffs namely carbohydrates, fats and proteins. The energy is utilized as follow. A considerable amount of energy is converted to heat and is utilized to maintain the body temperature; some portion of energy is utilized for the performance of work like muscular contraction, secretory function and nerve impulse conduction. Still some more amount of energy is stored temporarily in the high-energy phosphate bonds and stored for a longer period in the form of fat and glycogen to provide energy whenever required (Ambika, 2004). In crustaceans, assimilated energy is channelized into maintenance of metabolism (R) and production that includes growth assimilation (P) exuvia (E) generation and reproductive activity (Mootz and Epifanio, 1974; Levine and Sulkin, 1979). The energy expended in metabolic processes, measured by oxygen consumption, is used for the maintenance of physiological functions including locomotion, feeding, food processing, and for the synthesis of new tissue (Kiorboe and Mohlenberg, 1987). Growth (P) may be considered the energy materially gained by the individual and can be stored as body reserves. The partition of ingested energy into growth, metabolism, excretion and faeces may vary among different fishes and crustacean species depending on factors such as dietery composition (Cui *et al*., 1992), feeding (Odinetz-Collard *et al*., 1994) and food ration (Han *et al*., 2004). A balanced energy budget is a tool for bioenergetics modelling in aquaculture and fisheries management (Jobling, 1993). In the present study, following bio-energetic parameters were calculated to evaluate the energy budget/utilization by experimental prawns.

Feeding Rate

The growth rate of animals depends upon their feeding. Feeding rate is important for the growth, feed conversion, nutrient retention efficiency and chemical composition of body tissue (Storebakken and Austreng, 1987a, b). Determination of the nutrient requirement is also affected by feeding rate (Tacon and Cowey, 1985). A restricted feeding rate will cause impaired health (Storebakken and Austreng, 1987a) or slow growth (Hung and Lutes, 1987; Hung *et al*., 1989). The rate of feeding calculated revealed that, the control group fed minimum (0.412±0.018 k.cal/g/day), followed by 0.5%, 1%, 1.5% and 2% diets (0.453±0.010, 0.477±0.021, 0.477±0.036 and 0.513±0.044 k.cal/g/day). The statistical analysis made on the feeding rate between control and other diets revealed that, the variation was statistically significant (F=6.05, P<0.05). Similarly, Immanuel *et al*. (2003) reported that the probioitics *Lactobacillus* and yeast supplemented diets had improved the feeding rate of pearl spot *Etroplus suratensis*.

Absorption Rate

Part of the food ingested is assimilated in the gut and the remaining fraction is eliminated as faeces. The amount of food assimilated is dependent on the gut content and assimilation efficiency (Franco *et al*., 2006). In the present study, the amount of food absorbed in 60 days of the experimental period was maximum (0.547±0.040 K.cal/g/day) in 2% Binifit™ supplemented diet and those fed with control diet absorbed the minimum of 0.386±0.033 k.cal/g/day followed by the prawns

fed with 0.5% Binifit™ (0.430±0.025 K.cal/g/day), 1% Binifit™ (0.456±0.020 K.cal/g/day) and 1.5% Binifit™ (0.495±0.029 K.cal/g/day) (Table-3). One way ANOVA showed that, the variance in absorption rate among the diets was statistically significant (F=7.58, P<0.05). Similarly, Immanuel *et al*. (2003) reported that the probioitics *Lactobacillus* and yeast supplemented diets had improved the absorption of pearl spot *Etroplus suratensis*. Probiotic influences the digestive processes by enhancing the population of beneficial microorganisms, microbial enzyme activity; improving the intestinal microbial balance, consequently improving the digestibility and absorption of food and feed utilization (Bomba *et al*., 2002).

Conversion Rate

The feed conversion rate is one of the important parameters of feed quality. The Conversion rate is expressed as a ratio between food consumed for increase per unit weight gained by the body discounting the food energy requirement by the for its maintenance and energy requirement (Piska and Naik, 1999). The control diet has lowest conversion rate of 0.119±0.013 K.cal/g/day and those fed with 2% Binifit™ supplemented diet has highest conversion rate of 0.224±0.021 K.cal/g/day. Whereas it was 0.186±0.023, 0.166±0.020 and 0.142±0.024 K.cal/g/day in 1.5%, 1% and 0.5% diets respectively (Table-3). One way ANOVA showed that, the variance in conversion rate among the diets was statistically significant (F=10.80, P<0.05). Similarly, Immanuel *et al*. (2003) reported that the probioitics *Lactobacillus* and yeast supplemented diets had improved the conversion rate of pearl spot *Etroplus suratensis*.

NH_3 excretion rate

Ammonia excretion rate can serve as a good indicator for the optimum dietary protein content, especially when combined with data on growth rate. This approach looks promising for determining protein requirements, which can reduce dietary costs and minimize the nitrogenous waste output (Li Du and Cui-Juan Niu, 2002). The NH_3 excretion rate of *M. rosenbergii* fed with control diet was minimum (0.007±0.001 K.cal/g/day), followed by 0.5% (0.009±0.002 K.cal/g/day), 1% (0.011±0.001 K.cal/g/day), 1.5% (0.012±0.02 K.cal/g/day) and 2% Binifit™ (0.014±0.003 K.cal/g/day) supplemented diets fed groups (Table-3). The one-way ANOVA showed that, the variance in conversion rate among he diets was statistically significant (F=5.76, P<0.05). Similarly, Immanuel *et al*. (2003) reported that the probioitics *Lactobacillus* and yeast supplemented diets had improved the NH_3 excretion rate of pearl spot *Etroplus suratensis*.

Metabolic Rate

Metabolism is the set of chemical reactions that happen in living organisms to maintain life. These processes allow organisms to grow and reproduce, maintain their structures, and respond to their environments. The increase in metabolism after feeding is called specific dynamic action (Li Du and Cui-Juan Niu, 2002). It is also a major component in the energy budget of fish and has been reported to be dependent on several non-dietary factors, including body weight, density, and water temperature (Cho and Kaushik, 1985; Medland and Beamish, 1985), as a consequence of their influence on the overall metabolism of fish and other aquatic animals (Brett and Groves, 1979). In the present study, the *M. rosenbergii* fed with control diet has minimum metabolic rate of 0.274±0.031 K.cal/g/day and displayed the maximum metabolic rate of 0.337±0.024 K.cal/g/day in 2% Binifit™ incorporated diet fed group. In the remaining diets fed groups, it was 0.321±0.021 K.cal/g/day (1.5%), 0.301±0.036 K.cal/g/day (1%) and 0.297±0.025 K.cal/g/day (0.5%) respectively. One way ANOVA showed that, the variance in metabolic rate among the diets was statistically non significant (F=2.23, P>0.05). Similarly, Immanuel *et al*. (2003) reported that the probioitics *Lactobacillus* and yeast supplemented diets had improved the metabolic rate of pearl spot *Etroplus suratensis*. Here all the probiotic-supplemented diets resulted in enhanced feeding rate, absorption rate, conversion rate, NH_3 excretory rate and metabolic rate. From these findings, it is understood that the probiotics increased the energy budget performance of *M. rosnebrgii* PL.

From above discussion, it may be concluded that Binifit™ incorporation has significantly improved the survival, growth, SGR, FCR, PER, FCE, biochemical constituents and energy budget in post larvae of *M.rosenbergii*. Therefore, Binifit™ can be supplemented in formulated diets for healthy maintenance of *M. rosenbergii* at nursery and grows out pond. This can also be utilized for on farm feed management at small scale level and thus, inland aquaculture of *Macrobrachium* may be promoted in a sustainable manner.

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Table 1 Ingredients and proximate composition of experimental diets

S. No	Ingredients (g/kg^{-1})	Experimental Diets				
		Control	0.5% Binifit^{IM}	1% Binifit^{IM}	1.5% Binifit^{IM}	2% Binifit^{IM}
1	Fish meal	33.84	33.84	33.84	34.84	35.84
2	Ground nut oil	25.00	25.00	25.00	25.00	24.00
3	Soybean meal	24.00	24.00	24.00	22.50	22.00
4	Corn flour	4.00	3.50	3.00	3.00	3.00
5	Egg albumin	5.06	5.06	5.06	5.06	5.06
6	Topica flour	5.10	5.10	5.10	5.10	5.10
7	Cod liver oil	2.00	2.00	2.00	2.00	2.00
8	Vitamin mix	1.00	1.00	1.00	1.00	1.00
9	probiotics	0	0.5	1	1.5	2
Total		100	100	100	100	100
Proximate composition						
1	Protein (%)	45.69	45.08	45.02	45.06	44.95
2	Carbohydrate (%)	21.76	21.10	20.71	20.01	20.00
3	Lipid %)	5.70	5.70	5.51	5.27	5.05
4	Ash (%)	14.00	13.00	12.00	13.00	14.00
5	Moisture (%)	9.50	9.90	9.40	9.10	9.10
6	Digestible energy ($k.cal/kg^{-1}$)	3296.86	3296.86	3262.52	3262.52	3228.17

Table 2 Water stability of experimental diets in different hours

Diets	4 hours			6 hours			8 hours		
	Initial (g)	Final (g)	Leaching (%)	Initial (g)	Final (g)	Leaching (%)	Initial (g)	Final (g)	Leaching (%)
Control	1.00	0.910± 0.06	9.00	1.00	0.883± 0.04	11.70	1.00	0.863± 0.06	13.70
0.5% Binifit™	1.00	0.908± 0.07	9.20	1.00	0.880± 0.08	12.60	1.00	0.860± 0.05	14.00
1% Binifit™	1.00	0.904± 0.05	9.60	1.00	0.878± 0.07	12.20	1.00	0.857± 0.04	14.30
1.5% Binifit™	1.00	0.901±0.06	9.90	1.00	0.874± 0.05	12.00	1.00	0.855± 0.06	14.50
2% Binifit™	1.00	0.900± 0.04	10.00	1.00	0.870± 0.03	13.00	1.00	0.850± 0.09	15.00

Each value is a mean ± SD of three individual observations.

Table 3 The growth performance, biochemical constituents and energy budget of *M. rosenbergii* PL fed with different concentration of probiotics (Binifit™) supplemented diet.

parameters	Control diet	Experimental diets				F-value
		0.5% Binifit™	1% Binifit™	1.5% Binifit™	2% Binifit™	
Survival (%)	75.00±2.50^{d}	80.00±2.50^{c}	85.00±2.50^{b}	85.00±2.50^{b}	90.00±2.50^{a}	15.60
Weight gain (g)	0.60±0.06^{d}	0.70±0.05^{cd}	0.80±0.10^{bc}	0.88±0.07^{b}	1.04±0.10^{a}	13.78
Specific growth rate (%)	0.669±0.034^{d}	0.726±0.030^{c}	0.777±0.029^{bc}	0.814±0.032^{b}	0.880±0.026^{a}	21.35
Food conversion ratio (g)	3.18±0.50^{a}	2.75±0.27^{b}	1.88±0.24^{c}	1.77±0.20^{c}	1.57±0.30^{c}	19.93
Food conversion efficiency (%)	0.94±0.02^{e}	1.06±0.05^{d}	1.17±0.04^{c}	1.26±0.03^{b}	1.44 ±0.10^{a}	88.25
Protein efficiency rate (g)	0.826±0.042^{c}	0.941±0.032^{bc}	1.04±0.18^{abc}	1.12±0.19^{ab}	1.28±0.14^{a}	4.93
Protein (%)	58.00± 2.40^{b}	60.10± 2.80^{ab}	61.40± 2.24^{ab}	62.38± 2.14^{a}	64.12± 2.60^{a}	2.68
Amino acid (%)	26.80± 1.64^{c}	33.42± 1.48^{b}	35.32± 1.24^{ab}	37.26± 1.98^{a}	38.10± 1.64^{a}	23.37
Carbohydrate (%)	15.08± 1.02^{c}	16.19± 1.32^{bc}	18.33± 1.24^{ab}	19.38± 1.52^{a}	20.42± 1.60^{a}	6.02
Lipid (%)	7.82± 1.74^{c}	9.60± 1.28^{abc}	10.63± 1.32^{ab}	12.10± 1.18^{ab}	13.02± 1.64^{a}	8.01
Ash (%)	12.20± 1.20^{c}	14.60 ± 1.40^{bc}	16.40 ± 1.60^{ab}	17.90 ± 1.80^{a}	19.80 ± 2.00^{a}	7.20
Moisture (%)	77.40± 3.00^{a}	77.10± 2.80^{a}	77.00± 2.90^{a}	76.50± 3.20^{a}	75.00± 2.60^{a}	<1
Feeding rate (k.cal/g/day)	0.412± 0.018^{c}	0.453±0.010b^{c}	0.477±0.021^{ab}	0.495±0.029^{ab}	0.547±0.040^{a}	6.05
Absorption rate (k.cal/g/day)	0.386±0.033^{c}	0.430±0.025^{bc}	0.456±0.020^{ab}	0.477±0.036^{ab}	0.513±0.044^{a}	7.58
Conversion rate (k.cal/g/day)	0.119±0.013^{d}	0.142±0.024^{cd}	0.166±0.020^{bc}	0.186±0.023^{ab}	0.224±0.021^{a}	10.80
NH_3 Excretory rate (k.cal/g/day)	0.007±0.001^{c}	0.009±0.002^{bc}	0.011±0.001^{ab}	0.012±0.002^{ab}	0.014±0.003^{a}	5.76
Metabolic rate (k.cal/g/day)	0.274±0.031^{b}	0.297±0.025^{ab}	0.301±0.036^{ab}	0.321±0.021^{ab}	0.337±0.024^{a}	2.23

Each value is a mean ± SD of three replicate analysis, within each row means with different superscripts letters are statistically significant P<0.05 (one way ANOVA and subsequently *post hoc* multiple comparison with DMRT).